High-Voltage Electric Discharge Extraction of Bioactive Compounds from the Cocoa Bean Shell+

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This study is focused on the application of high-voltage electric discharge (HVED) to recover some bioactive compounds from the cocoa bean shell. Different extraction times (30, 60, 90 min), frequencies (40, 70, 100 Hz) and solvent-solid ratios (10, 30 and 50 mL g^{-1}) were used to obtain cocoa bean shell extracts. Desired bioactive compounds, methylxanthines and phenolic compounds were measured in obtained extracts by high-pressure liquid chromatography with diode array detector. The obtained extracts showed that theobromine was the most abundant, ranging from 2530.13 to 6031.51 mg kg^{-1}, while caffeine content was in the range from 316.08 to 849.88 mg kg^{-1}. In addition, significant amounts of phenolic compounds were found, namely catechin (115.91 to 284.33 mg kg^{-1}), epicatechin (20.20 to 358.90 mg kg^{-1}), and gallic acid (80.28 to 219.17 mg kg^{-1}). Results showed that different parameters of HVED extraction have statistically significant influence on cocoa bean shell composition, suggesting how this by-product can be used in the production of valuable extracts.

Keywords:
cocoa bean shell, by-product, high-voltage electric discharge, bioactive compounds

Introduction

Over the last decades, positive changes in analytical chemistry have been made, cutting down the use of toxic chemicals and reducing their influence on the environment due to Green Analytical Chemistry trends. Accordingly, the development of innovative sustainable green extraction techniques have become more interesting due to the cleaner, greener, and safer nature of these processes and easier usage[1]. Thus, modern extraction techniques have been introduced and are being applied more and more for extraction of various materials and compounds. Some of these techniques are: supercritical fluid extraction (SFE), subcritical water extraction (SWE), superheated water extraction or pressurized hot water extraction (PHWE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE)[2].

One of these eco-friendly techniques, which gain more and more attention in the last years, is also high-voltage electrical discharges (HVED), a non-thermal technology suitable for processes where high temperatures are undesirable. This technique enhances the yield of bioactive compounds from raw material at low treatment energy input[3]. Detailed description of the HVED process is given in the review published by Boussetta and Vorobiev[4] where authors pointed out that HVED can be applied in numerous applications, particularly in the extraction of various bioactive compounds. Li *et al.*[5] in their recently publish review, pointed out that critical process factors for HVED-assisted extraction are electric field intensity, flow rate, solvent-solid ratio, treatment time, and solvent selection. The authors concluded how the further development of HVED-assisted extraction will definitely be a benefit in the future. Sarkis *et al.*[6] investigated two different electrical technologies, pulsed electric fields (PEF) and HVED as pre-treatments to sesame seed oil extraction. The authors compared both procedures to a control sample, and concluded that treated samples had higher oil yield in comparison to controlled samples. Bousetta *et al.*[7] explored the effect of HVED on the aqueous extraction of polyphenols from grape pomace, and obtained bet-

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ter yields than without HVED treatment. They indicated HVED treatment as useful for reducing both extraction time and temperature. In another study, Bousetta *et al.*[8] investigated the effect of HVED extraction of lignans and polyphenols from whole and crushed flaxseed cake with water and ethanol addition. The authors stated that HVED treatment was an effective technique for disrupting plant tissues and improving the release of intracellular compounds. Roselló-Sotto *et al.*[9] discovered that HVED is more effective than ultrasound or PEF in extraction of specific compounds like phenolics and proteins from olive kernel. Brianceau *et al.*[10] investigated the influence of HVED on extraction of phenolic compounds from grape stems and discovered significantly improved extraction of flavan-3-ols and flavonols, which was not the case with stilbenes.

From the above, it is evident that HVED can also be used for the treatment of food industry by-products in the extraction of bioactive compounds. Cocoa bean shell (CBS) is one of those by-products from the production of cocoa and its products, rich in specific bioactive compounds[11]. CBS makes 67 % of total waste in the cocoa industry that has been discarded for years[12]. In the fermentation stage, during the processing of cocoa beans, methylxantines migrate from the bean into the CBS[13] as do some polyphenols[14]. According to the beneficial effects of cocoa on human health due to its polyphenol content, antioxidant and anti-inflammatory properties as well as contributions to normal blood flow, it would be wise to recover those compounds and use them in the production of new functional food[15]. Out of all bioactive compounds contained in CBS, theobromine prevails. This colorless, odorless, and slightly bitter tasting substance is contained in all parts of the seed and serves as a chemical defense mechanism of the cocoa plant. It contributes to the typical bitter taste of cocoa and its products. Although it is considered toxic in larger quantities, in small doses it has numerous pharmacological activities, such as anticancer, diuretic, cardiac stimulant, hypocholesterolemic, smooth-muscle relaxant, anti-asthma and coronary vasodilator[16]. The next most abundant methylxantine present in CBS is caffeine, a stimulant with positive effects on central nervous system as well as on the gastrointestinal, vascular and respiratory systems[13,17]. The presence of theophylline as the third methylxanthine in CBS is negligible[13].

From all previously mentioned, the aim of this study was to determine the impact of the HVED process on the extraction of bioactive compounds in CBS (first time report), which ultimately can result in production of enriched CBS extracts with further usage as functional food products.

Material and methods

Material and chemicals

CBS was obtained from chocolate factory Kandit d.o.o., Osijek, Croatia, in summer 2017. The countries of origin of CBS were Ghana and the Ivory Coast. CBS samples were obtained by roasting fermented cocoa beans at 135 °C for 55 minutes. The cocoa shell was then easily separated from the cotyledon.

All used chemicals, including standards and organic solvents were of analytical grade. Solvents were purchased from J. T. Baker (PA, USA). The theobromine standard (purity ≥98 %), gallic acid (purity ≥99 %), epicatechin (purity ≥98 %), and catechin (purity ≥99 %) were purchased from Sigma Aldrich (Germany), while caffeine standard (≥98 %) was purchased from Dr. Ehrenstorfer (Germany).

High-voltage electric discharge extraction of CBS

The experiment was carried out with a custom-made and automatized HVED device at the Faculty of Food Technology Osijek (Croatia). High-voltage generator had working voltage of 30 kV and maximum electric current of 10 mA. Maximum electric power of the DC generator was 120 W. Capacitor stored the energy released in the form of a discharge within the chamber. High-voltage switch enabled discharge from the capacitor to the chamber, at specified intervals. The intervals between discharges defined the frequency, which was adjusted from 10 Hz to 100 Hz. The sample entered the chamber, where the discharge was between electrodes inserted in the sample, with pin-to-plate type. The reaction was enhanced by stirring with a magnetic stirrer.

Before the HVED extraction, CBS was ground using the laboratory mill and sieved for 20 minutes using a vertical vibratory sieve shaker (Labortechnik Gmbh, Ilmenau, Germany). The average particle size was determined to be 0.296 mm +/– 0.088[18]. All measurements were made in triplicate. HVED extraction was carried out under different conditions of time (30, 60, 90 min), frequency (40, 70, 100 Hz), and solvent-solid ratio (10, 30 and 50 mL g^{-1}) given in Table 1, according to Box-Behnken Design (BBD) explained in more details in our previous paper[19].

In order to evaluate the impact of HVED on bioactive compounds of CBS, the control samples were prepared. The control samples represented water extracts of CBS prepared at equal conditions of solid-solvent ratio of 10, 30 and 50 mL g^{-1}, and mixed in magnetic stirrer for 30, 60 and 90 minutes.

Table 1 – *Coded and real levels of independent variables for the designed experiment*

Independent variable	Symbol	Level		
		Low (–1)	Middle (0)	High (+1)
Solvent-solid ratio (mL g^{-1})	X_1	10	30	50
Frequency (Hz)	X_2	40	70	100
Time (min)	X_3	30	60	90

Determination of bioactive compounds in CBS extracts by HPLC

Identification and quantification of bioactive compounds in CBS extracts was done according to Pura Naik[20] study with slight modifications. The sample extracts obtained from HVED extraction were filtered through 0.2 μm PTFE filter, ready for the chromatographic analysis. The measurement took place on reverse-phase High Performance Liquid Chromatography (HPLC) Infinity 1260 Agilent Technologies (USA) instrument containing an autosampler G7129A, quaternary pump G7111B 1260, and diode array detector (DAD) G7117C 1260 DAD HS. For HPLC analysis, Zorbax C_{18} 150 mm x 4.6 mm x 5μm column was used with temperature set at 30 °C. The wavelength was set to 276 nm and the injection volume was 20 μL. Mobile phase was gradient, starting with 1 % formic acid and acetonitrile (95:5), and changing to (80:20) for 9 minutes, and returning to (95:5) for 13 minutes, which was also the analysis run time. The flow of the mobile phase was 1 mL min^{-1} and the analyses were done in triplicate.

Determination of total phenolic content (TPC) and DPPH scavenging activity

Immediately after each extraction process, the total phenolic content (TPC) as well as DPPH scavenging activity of obtained extracts were analyzed. TPC of CBS extracts was determined by modified spectrophotometric method using Folin-Ciocalteu reagent. The results were expressed in mg of gallic acid equivalents (GAE) per g of the extracts. The measurements were performed in triplicate.

Antioxidant activity of obtained CBS extracts was determined by DPPH scavenging described in detail in our earlier published paper[21]. The measurements were performed in triplicate.

Statistical analysis

Experimental data were statistically analyzed using the commercial Design-Expert® software (ver. 9, Stat-Ease Inc., Minneapolis, MN, USA). The analysis of variance (ANOVA) was used to estimate the quality of the model. The test of statistically significant difference was based on the total error criteria with the level of confidence of 95.0 %. The response plots were generated using the same software for better understanding of the correlation of independent and response variables.

Results and discussion

Bioactive compounds of CBS extracts

Based on our previous work[19], we assumed the possible bioactive compounds in CBS extracts (methylxantines: theobromine, theophylline, and caffeine; phenolic compounds: gallic acid, catechin, epicatechin, epigallocatechin, caffeic acid, chlorogenic acid, vanillin; and 5-hydroxymethylfurfural, respectively). From these eleven analyzed bioactive compounds, five were identified and quantified in HVED extracts (theobromine, caffeine, catechin, epicatechin, and gallic acid). The other compounds were not present in the samples of CBS extracts or were below the detection limit, and were not presented in tables. The extraction of control samples (Table 2) was performed and compared with HVED samples (Table 3) under the same extraction conditions to evaluate the real effect of high voltage. Of all analyzed bioactive compounds, theobromine was extracted in the highest amount, followed by caffeine, catechin, epicatechin, and gallic acid.

Comparison of the bioactive compounds obtained in control samples with those obtained by HVED extraction, suggested a noticeable increase, especially in theobromine and epicatechin content in extracts obtained by HVED. This demonstrated the efficiency of the HVED extraction in the recovery of tested bioactive compounds from CBS.

In our previous study, where subcritical water extraction was applied[19], presence of 5-hydroxymethylfurfural in CBS extracts was observed only at higher extraction temperature, while extraction using HVED produced no 5-hydroxymethylfurfural, which was good considering the characteristics and nature of this compound. Although it may be metabolized to 5-sulfoxymethylfurfural, which has antioxidative and anti-inflammatory properties, 5-hydroxymethylfurfural is linked to some detrimental effects due to its mutagenic, genotoxic, organotoxic properties[22]. Therefore, it may be said that, in the best case, its safety is questionable.

Methylxantines in CBS extracts

Similar to flavonoid compounds, methylxantines also migrate from cocoa bean to the CBS during fermentation process and decrease theobro-

Table 2 – *Bioactive components detected in control samples*

RUN	Solvent-solid ratio (mL g^{-1})	Time (min)	Gallic acid (mg kg^{-1})	Theobromine (mg kg^{-1})	Catechin (mg kg^{-1})	Caffeine (mg kg^{-1})	Epicatechin (mg kg^{-1})	TPC (mg GAE g^{-1})	DPPH (%)
1	30	30	110.79	2638.32	140.45	534.91	128.90	86.74	50.68
2	30	60	123.66	2608.47	104.32	529.79	118.98	85.97	50.96
3	50	60	113.90	2961.13	138.85	602.81	124.84	119.31	53.69
4	30	90	111.82	2706.55	169.34	587.04	88.45	85.72	56.86
5	10	60	94.63	1682.69	170.34	365.07	133.95	76.23	47.47
6	50	30	105.02	2726.12	174.76	563.63	105.83	71.62	52.18
7	50	90	114.31	2988.67	187.53	678.44	109.33	87.00	53.04
8	10	90	107.82	1754.18	123.68	351.78	140.02	79.05	50.68
9	10	30	66.32	1692.10	152.13	344.51	96.80	68.28	45.73

Table 3 – *Bioactive components detected in CBS extracts obtained by HVED*

RUN	Solvent-solid ratio (mL g^{-1})	Frequency (Hz)	Time (min)	Gallic acid (mg kg^{-1})	Theobromine (mg kg^{-1})	Catechin (mg kg^{-1})	Caffeine (mg kg^{-1})	Epicatechin (mg kg^{-1})	TPC (mg GAE g^{-1})	DPPH (%)
1	30	40	30	170.69	4294.54	169.50	574.49	358.90	69.05	39.90
2	30	70	60	163.98	4331.82	208.89	583.52	229.08	65.21	38.96
3	30	70	60	160.82	4383.95	185.99	602.80	215.28	70.08	36.59
4	30	100	30	219.17	5246.36	284.33	752.32	270.13	72.39	40.37
5	30	70	60	80.28	4343.55	115.91	608.37	155.28	67.00	29.83
6	30	70	60	95.53	4331.44	132.76	616.96	132.65	77.26	41.91
7	30	70	60	143.01	4159.31	203.96	542.27	159.62	66.74	35.69
8	50	40	60	91.08	4684.98	147.11	660.22	20.20	69.05	39.53
9	30	100	90	112.76	2865.90	198.99	402.68	133.73	75.46	41.44
10	50	100	60	90.66	6031.51	154.69	849.88	125.31	92.39	45.45
11	10	40	60	147.32	3478.14	283.45	437.68	226.30	60.85	27.69
12	10	100	60	115.97	3750.48	184.73	468.32	211.72	59.82	27.53
13	30	40	90	145.07	3866.84	200.46	508.82	81.21	77.51	42.24
14	50	70	30	125.31	4581.06	197.78	619.83	75.08	94.95	50.97
15	50	70	90	126.24	4102.99	203.86	558.53	93.23	78.03	44.42
16	10	70	90	145.95	3479.19	136.47	431.49	218.39	58.29	25.55
17	10	70	30	84.30	2530.13	167.77	316.08	159.24	52.39	24.92

mine content by about 25 % in cocoa beans[23]. Of all analyzed compounds in CBS extracts, theobromine and caffeine were the most prevalent. We obtained the same conclusion in our previously published study[19] where we examined the influence of subcritical water extraction process on the content of bioactive compounds in CBS.

In this study, the HVED extraction conditions at frequency of 100 Hz for 60 minutes using solvent-solid ratio of 50 mL g^{-1}, gave the best yields for two dominant methylxantines (theobromine 6031.52 mg kg^{-1}, and caffeine 849.88 mg kg^{-1}), while theophylline was not detected. The lowest yields for those compounds were at 70 Hz during 30 minutes using solvent-solid ratio 10 mL g^{-1} (2530.13 mg kg^{-1} for theobromine, and 316.08 mg kg^{-1} for caffeine). Since different types of extractions had significant influence on the composition of the final extracts, it is not surprising that amounts of determined compounds varied according to applied extraction method and parameters. For example, Hernández-Hernández *et al.*[24] provides results for raw CBS (3900 mg theobromine kg^{-1}) and fermented CBS (12000 mg theobromine kg^{-1}) representing the strong influence of fermentation on the appearance of those and other bioactive com-

pounds in CBS samples (caffeic acid, catechin, epicatechin and epigallocatechin). Another study showed a different proportion of theobromine and caffeine in CBS obtained by pulsed electric field assisted extraction and depending on the cocoa origin (range for theobromine: 4640 – 10920 mg kg^{-1}, and for caffeine 1590 – 4210 mg kg^{-1})[17]. Adamafio[25] pointed out that cocoa from Africa has the highest proportion of theobromine, and gave a range of concentrations for theobromine in CBS from 5000 to 21000 mg kg^{-1}, also depending on the origin of the same raw material. Okiyama *et al.*[26] also confirmed the presence of theobromine in CBS as the main compound in this by-product (9890 mg kg^{-1}) using pressurized liquid extraction. To the best knowledge of the authors, the application of HVED for extraction of bioactive compounds from CBS is not available in scientific literature, so the obtained results of this study could not be compared.

Phenolics, total phenol content, and antioxidant activity of CBS extracts

Hernández-Hernández *et al.*[24] mentioned epicatechin and catechin as the most prevalent phenolic compounds in cocoa beans, together with their transition to CBS after the fermentation process, which makes CBS an excellent natural source of these compounds, especially epicatechin. Flavonoid compounds, catechin and epicatechin, have strong antioxidant activity with ability to decrease oxidative stress[27] and improve cardiovascular function[28]. Best yields of phenolic compounds were obtained by extraction at 100 Hz during 30 minutes using solvent-solid ratio 30 mL g^{-1} (catechin 284.33 mg kg^{-1}, epicatechin 270.13 mg kg^{-1}, and gallic acid 219.17 mg kg^{-1}), while even higher concentrations of epicatechin were obtained using following extraction conditions: frequency 40 Hz, 30 minutes, and 30 mL g^{-1} (358.90 mg kg^{-1}). The obtained results of HVED extraction show a strong correlation between TPC and antioxidant activity. Jokić *et al.*[19] also showed a significant correlation between TPC and DPPH scavenging activity for CBS obtained by subcritical water extraction (130.33 mg GAE g^{-1} extract and 91.69 %) at temperature 220 °C, time 75 minute, and 20 mL g^{-1} solvent-solid ratio. In this study, the lowest values for TPC and DPPH, 52.39 mg GAE g^{-4} of extracts and 24.92 % DPPH scavenging activity, were obtained under extraction conditions of 70 Hz, 30 minutes, and 10 mL g^{-1}. Such different results for TPC and antioxidant activity indicate that they strongly depend on the applied conditions of HVED extraction, as well as the type of extraction. Another study also showed strong antioxidant activity of phenolic compounds discovered in CBS extracted with methanol[29]. Martinez *et al.*[30] showed that methanol:acetone extraction mixture gave a significantly better TPC (between 144.83 and 154.43 mg GAE/100 g) than extraction with ethanol (between 80.17 and 82.37 mg GAE/100 g), depending on the locality of the species. Xi, He and Yan[31] optimized HVED for the first time to extract phenolic compounds from pomegranate peel, made a correlation to warm water maceration, and indicated the advantage of the HVED extraction method due to higher efficiency of extraction of phenolic compounds. Mazzuti *et al.*[32] emphasized that antioxidants can be extracted effectively and more rapidly from CBS by integrated green-based process in comparison with conventional Soxhlet extraction, also suggesting that this byproduct could be a valuable source of bioactive compounds for various applications in the food, cosmetic, pharmaceutic or biomedical industries.

Response surface methodology and optimization

Optimization is the fundamental tool in food engineering processes for the productive operation of different processes to gain a valuable and acceptable product[33]. In order to optimize the most important operating variables for HVED extraction of CBS (time, frequency and solvent-solid ratio), and to achieve the highest amount of targeted bioactive compounds in extracts, the response surface methodology (RSM) and BBD were used. In Table 4, regression coefficients together with their *p*-values are given for the most abundant compounds. From the obtained coefficients for each response, the models can be created and used for simulation of the HVED process, which is the final goal of RSM.

Developed second-order polynomial models (in terms of coded values) for prediction of targeted compounds/responses (theobromine content, caffeine content, total phenols, and antioxidant activity) in CBS extracts are given in Eqs. 1–4:

$$y_1 = 4309.99 + 770.33X_1 + 196.22X_2 - 292.15X_3 - 109.39X_1^2 + 285.67X_2^2 - 527.26X_3^2 + 268.55X_1X_2 - 356.78X_1X_3 - 488.19X_2X_3 \quad (1)$$

$$y_2 = 590.78 + 129.36X_1 + 36.50X_2 - 45.15X_3 - 32.43X_1^2 + 45.67X_2^2 - 76.88X_3^2 + 39.76X_1X_2 - 44.18X_1X_3 - 70.99X_2X_3 \quad (2)$$

$$y_3 = 69.26 + 12.88X_1 + 2.95X_2 + 0.064X_3 - 0.71X_1^2 + 1.98X_2^2 + 2.37X_3^2 + 6.09X_1X_2 - 5.71X_1X_3 - 1.35X_2X_3 \quad (3)$$

$$y_4 = 36.6 + 9.34X_1 + 0.68X_2 - 0.31X_3 - 3.03X_1^2 + 1.49X_2^2 + 2.9X_3^2 + 1.52X_1X_2 - 1.8X_1X_3 - 0.32X_2X_3 \quad (4)$$

Table 4 – *Regression coefficients of the polynomial function of theobromine and caffeine content, total phenolic content and DPPH assay*

Term	Coefficients	Standard error	*F*-value	*p*-value
		Theobromine		
Intercept	4309.99	227.08		
X_1	770.33	179.52	18.41	0.0036
X_2	196.22	179.52	1.19	0.3106
X_3	–292.15	179.52	2.65	0.1477
X_1^2	–109.39	247.45	0.2	0.6718
X_2^2	285.67	247.45	1.33	0.2862
X_3^2	–527.26	247.45	4.54	0.0706
X_1X_2	268.55	253.88	1.12	0.3253
X_1X_3	–356.78	253.88	1.97	0.2027
X_2X_3	–488.19	253.88	3.7	0.0959
		Caffeine		
Intercept	590.78	32.78		
X_1	129.36	25.91	24.92	0.0016
X_2	36.50	25.91	1.98	0.2018
X_3	–45.15	25.91	3.04	0.1250
X_1^2	–32.43	35.72	0.82	0.3941
X_2^2	45.67	35.72	1.63	0.2418
X_3^2	–76.88	35.72	4.63	0.0684
X_1X_2	39.76	36.65	1.18	0.3139
X_1X_3	–44.18	36.65	1.45	0.2672
X_2X_3	–70.99	36.65	3.75	0.0939
		TPC		
Intercept	69.26	2.77		
X_1	12.88	2.19	34.51	0.0006
X_2	2.95	2.19	1.81	0.2207
X_3	0.064	2.19	$8.54\cdot10^{-4}$	0.9775
X_1^2	–0.71	3.02	0.055	0.8207
X_2^2	1.98	3.02	0.43	0.5333
X_3^2	2.37	3.02	0.61	0.4596
X_1X_2	6.09	3.10	3.85	0.0904
X_1X_3	–5.71	3.10	3.38	0.1085
X_2X_3	–1.35	3.10	0.19	0.6774
		DPPH		
Intercept	36.60	1.89		
X_1	9.34	1.49	39.17	0.0004
X_2	0.68	1.49	0.21	0.6635
X_3	–0.31	1.49	0.044	0.8395
X_1^2	–3.03	2.06	2.18	0.1835
$X_2X_3X_2^2$	1.49	2.06	0.52	0.4924
X_3^2	2.9	2.06	1.99	0.2009
X_1X_2	1.52	2.11	0.52	0.4940
X_1X_3	–1.8	2.11	0.73	0.4223
X_2X_3	–0.32	2.11	0.023	0.8845

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \\ + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$$

X_1: Solvent-solid ratio; X_2: frequency X_3: time

*Significant at $p \leq 0.05$

Table 5 – *Analysis of variance (ANOVA) for the response surface quadratic models*

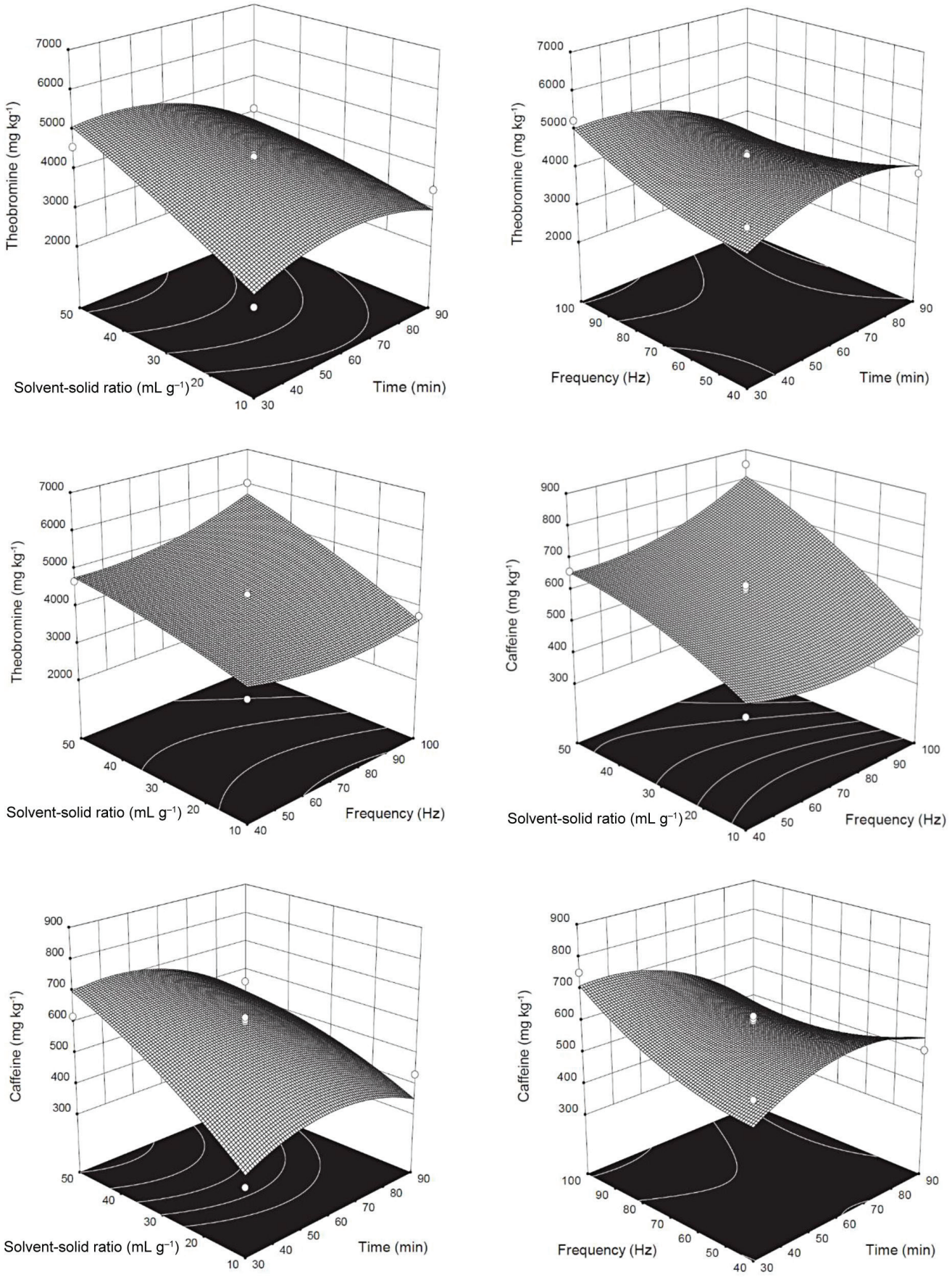
Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value
		Theobromine			
The recovery					
Model	$9.004\cdot10^6$	9	$1\cdot10^6$	3.88	0.0438*
Residual	$1.805\cdot10^6$	7	$2.578\cdot10^5$		
Lack of fit	$1.775\cdot10^6$	3	$5.915\cdot10^5$	78.26	0.0005
Pure error	30231.82	4	7557.95		
Total	$1.081\cdot10^7$	16			
		Caffeine			
The recovery					
Model	$2.323\cdot10^5$	9	25807.24	4.80	0.0253*
Residual	37604.11	7	5372.02		
Lack of fit	34058.44	3	11352.81	12.81	0.0161
Pure error	3545.66	4	886.42		
Total	$2.699\cdot10^6$	16			
		TPC			
The recovery					
Model	1726.69	9	191.85	4.99	0.0229*
Residual	269.38	7	38.48		
Lack of fit	176.89	3	58.96	2.55	0.1938
Pure error	92.49	4	23.12		
Total	1996.07	16			
		DPPH			
The recovery					
Model	804.05	9	89.34	5.02	0.0225*
Residual	124.58	7	17.80		
Lack of fit	44.21	3	14.74	0.73	0.5841
Pure error	80.37	4	20.09		
Total	928.63	16			

*Significant at $p \leq 0.05$

where y_1 is predicted response for theobromine content, y_2 for caffeine content, y_3 for TPC, and y_4 for DPPH values. X_1 to X_3 are coded values of input variables given in Table 1.

Table 5 shows the analysis of variance (ANOVA) for obtained models of methylxantines, TPC and DPPH. The statistical significance for each individual factor is represented by its *p*-value. The linear term of solvent-solid ratio statistically showed the most significant influence on all investigated responses ($p < 0.05$).

The data were used for the creation of 3D graphs of the selected response surfaces (Figs. 1–2), and the plots were gained by displaying two selected variables within the experimental range, while the third variable remained constant at its respective value in the experimental range. The graphs visualize the impact of process parameter as independent variables on theobromine content, caffeine content, and TPC, and antioxidant activity as dependent



F i g . 1 – *Response surface plots showing the effects of investigated variables on theobromine and caffeine content as a function of different process conditions*

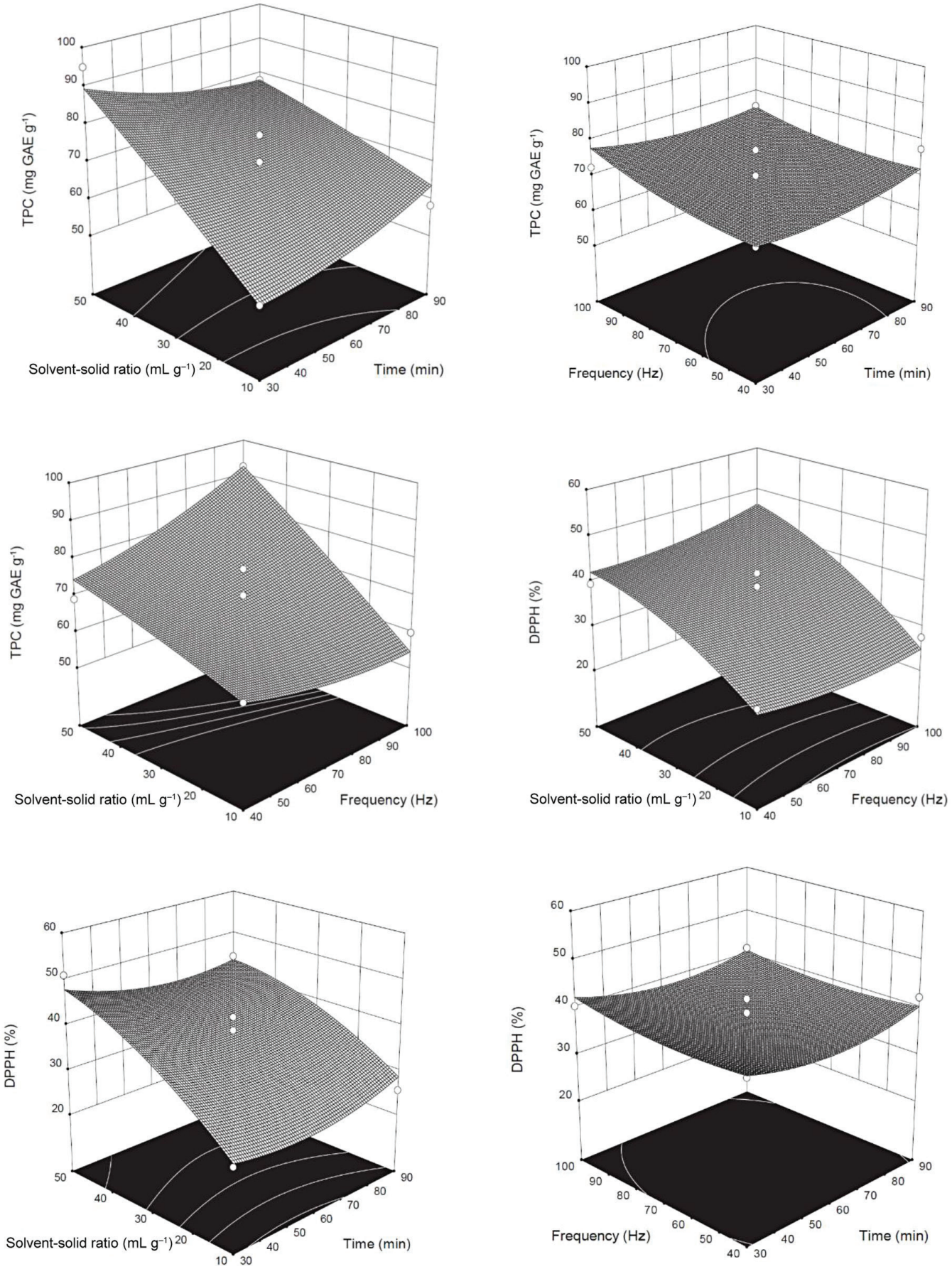


Fig. 2 – *Response surface plots showing the effects of investigated variables on TPC and DPPH scavenging activity as a function of different process conditions*

variables. The three-dimensional plots for methylxanthines (theobromine and caffeine) as the two most abundant bioactive compounds in obtained extracts showed very similar results and shapes where solvent-solid ratio had a significant influence on methylxantine concentrations in obtained extracts during HVED extraction. The plots show that by increasing solvent-solid ratio the content of these two bioactive compounds (Fig. 1) as well as TPC and antioxidant activity increase significantly (Fig. 2). In contrast, frequency and extraction time had no significant influence on methylxantine concentrations in obtained extracts as well as on TPC and DPPH. TPC and DPPH also showed similar response plot shapes, which was anticipated according to high positive correlation between TPC and antioxidant activity (R^2=0.911).

By applying desirability function method, and considering the maximum, the optimal conditions for HVED extraction of CBS were calculated to be at frequency 100 Hz, time 30 min, and solvent-solid ratio 47 mL g^{-1}.

Conclusion

Cocoa bean shell, as an accumulating waste in the cocoa industry, contains many bioactive compounds, such as methylxanthines theobromine and caffeine, and also phenolic compounds like catechin, epicatechin, and gallic acid. HVED, as an innovative non-thermal process, can be used in production of CBS extracts with a significantly higher amount of selected compounds, especially theobromine and epicatechin, which is proven by comparison with control samples under the same conditions. Those enriched CBS extracts with bioactive compounds could be suitable for later use as a raw material in other production processes, for example in production of functional food products.

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